EXTRACTION OF PHENOLIC COMPOUNDS AND FLAVONOIDS COMPOUNDS FROM ORTHOSIPHON STAMINEUS

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PENGESTRAKKAN SEBATIAN FENOLIK DAN SEBATIAN FLAVONOIDS DARI ORTHOSIPHON STAMINEUS

ABSTAK

Sebatian fenolik dan flavonoid terkandung dalam *Orthosiphon stamineus* (OS) mempamerkan kapasiti antioksidan yang tinggi. Kajian ini telah dijalankan untuk membandingkan pengekstrakan ultrasonik (UAE) dan microwave pengekstrakan (MAE) dengan maceration dan menyiasat kesan pelarut (70% -30%v/v metanol-air, 70% -30% v/v propanol-air, 50% -50% v/v metanol-air, 50% -50% v/v propanol-air, air, metanol dan propanol),masa pengekstrakan, kuasa microwave dan suhu pengekstrakan ke atas pengestrakkan Rosmarinic asid dan Sinensetin dari serbuk OS kering. Hasil telah dianalisis dengan menggunakan Ultra Performance Liquid Chromatography (UPLC) dan telah berbanding dengan penentukuran standard dengan masa penahanan mereka. Hasil daripada Jumlah kandungan fenolik (TPC) telah digunakan untuk menyokong hasil UPLC Dengan UAE, hasil tertinggi bagi Rosmarinic asid boleh diperolehi dengan syarat berikut:. 70% -30% v / v Metanol-air sebagai pelarut dengan masa pengekstrakan 90 minit pada 60 c.Hasil tertinggi untuk asid Rosmarinic yang boleh diperolehi dengan menggunakan MAE adalah dengan syarat-syarat berikut: 70% -30% v/v Metanol-air dengan masa pengekstrakan 120 s pada 450 W. DenganUAE, hasil tertinggi untuk Sinensetin boleh diperolehi dengan syarat berikut: propanol sebagai pelarut dengan masa pengekstrakan 90 minit pada 60 C Sementara itu, hasil tertinggi bagi Rosmarinic asid yang boleh diperolehi dengan menggunakan MAE adalah dengan syarat-syarat berikut, 70% -30% v / v Metanol-air dengan pengekstrakanmasa 120 s pada 450 W. UAE dan MAE boleh menggantikan maceration dalam pengekstrakan sebatian fenolik dan flavonoid sebatian kerana UAE dan MAE mempunyai hasil yang lebih tinggi dalam masa yang lebih singkat. Cadangan mencadangkan untuk mendapatkan pengoptimuman kajian ini adalah untuk mengkaji kesan yang lebih atau parameter dalam kedua-dua UAE dan MAE.

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ABSTRACT

Phenolic and flavonoids compounds present in *Orthosiphon stamineus* (OS) exhibit high antioxidant capacity. This research was conducted to compare ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) with maceration and investigate effects of solvents (70%-30% v/v methanol-water, 70%-30% v/v propanol-water, 50%-50% v/v methanol-water, 50%-50% v/v propanol-water, water, methanol and propanol), extraction time, microwave power and extraction temperature on Rosmarinic acid and Sinensetin extraction yields from OS dried powder. The extraction yields were analyzed by using Ultra Performance Liquid Chromatography (UPLC) and were compared to standard calibration curve with their retention times. Results from Total Phenolic Content (TPC) were used to support UPLC result. By UAE, the highest yield for Rosmarinic acid could be obtained with following conditions: 70%-30% v/v Methanol-water as solvent with extraction time of 90 minutes at 60˚C. The highest yield for Rosmarinic acid that could be obtained by using MAE was with following conditions: 70%-30% v/v Methanol-water with extraction time of 120 s at 450 W. By UAE, the highest yield for Sinensetin could be obtained with following conditions: propanol as solvent with extraction time of 90 minutes at 60˚C. Meanwhile, the highest yield for Rosmarinic acid that could be obtained by using MAE was with following conditions, 70%-30% v/v Methanol-water with extraction time of 120 s at 450 W. UAE and MAE can replace maceration in extraction of phenolic compounds and flavonoids compounds since UAE and MAE had higher yields than maceration in shorter time. Recommendations suggested in order to obtain optimization of this research were to study more effects or parameters in both UAE and MAE.

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GA	Gallic Acid
MAE	Microwave-Assisted Extraction
OS	*Orthosiphon stamineus*
RA	Rosmarinic Acid
Sin	Sinenesetin
TPC	Total Phenolic Content
UAE	Ultrasonic-Assisted Extraction
UPLC	Ultra-Performance Liquid Chromatography

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia's tropical climate contributes to tropical rainforest which supports more than 20000 plant species (Indu and Ng, 2000) and over 3000 herbs are identified as medicinal plants (Ramlan, 2009.) which are reported to have therapeutic and chemical properties. Herbal products had gained popularities and were commercialize sold in pharmaceutical dosage forms, such as capsules, tablets, pills, liquid preparations, creams, lotions, suppositories, patches (Jamal, n.d.) and also dietary supplements. One of the traditional herbs that had gained much attention was *Orthosiphon stamineus* or commonly known as "Misai Kucing" which was believed to have synergistic bio-enhancing ability for tamoxifen against human breast cancer antioxidant capacity according to Ahamed and Abdul in 2010. Besides, the leaves of *Orthosiphon stamineus* are commonly used as herbal tea or Java tea for diuresis, to treat rheumatism, diabetes, urinary lithiasis, oedema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension (Hossain and Mizanur Rhaman, 2011).

In Malaysia, the plant is used for wide range of diseases treatment such as eruptive fever, epilepsy, gallstone, hepatitis, hypertension, syphilis, and renal calculus (Akowuah *et al*., 2004). It had been used for kidney related and joint ailments such as gall stones, diabetes, arthritis, rheumatism and gout. It had been proven to remove uric acid through its diuretic activity which was the main path for its therapeutic activity (Ramlan *et al*., n.d.).

In the past, several techniques had been used for extraction of phenolic and flavonoids compounds from *Orthosiphon stamineus* such as Soxhlet extraction (Hossain and Mizanur Rahman, 2011), maceration (Mohammad *et al*., 2011), and reflux extraction (Matkwoski, 2008). Many types of solid-liquid extraction methods are available nowadays such as pressurized fluid extraction, microwave-assisted extraction, matrix solid phase dispersion and supercritical fluid extraction. Extraction of flavonoids and phenolic compounds from *Orthosiphon stamineus* is one of the most important steps prior to their determination by Ultra Performance Liquid Chromatography (UPLC).

This research was conducted to investigate the effects of solvent, extraction temperature, ultrasonic or microwave power and extraction time on total flavonoid content and total phenolic content of *Orthosiphon stamineus* leaves by three extraction methods which were maceration, ultrasonic-assisted extraction and microwave-assisted extraction.

1.2 Problem Statement

Conventional methods that had been used to extract phenolic and flavonoids compounds from *Orthosiphon stamineus* was maceration. In recent years, new methods had been developed in extraction technologies which were pressurized fluid extraction, microwave-assisted extraction, matrix solid phase dispersion, ultrasonic-assisted extraction and supercritical fluid extraction. However, the effects of solvent types, extraction temperature, extraction time and power on extraction yields on phenolic and flavonoid compounds were not fully investigated. So, this research was conducted to investigate these parameters in ultrasonic-assisted extraction, microwave-assisted extraction and maceration.

1.3 Research Objective

This research aimed to:

- extract phenolic and flavonoids compounds from *Orthosiphon stamineus*
- compare yield of phenolic and flavonoids compounds among three extraction methods which were ultrasonic-assisted extraction, microwave-assisted extraction and maceration
- determine the best operating parameter for phenolic and flavonoids compounds for each extraction method

1.4 Scope of Study

The scope of this research was to analyze main parameters which were types of solvent, extraction temperature, ultrasonic or microwave power and extraction time. Only *Orthosiphon staminues* was used in this research. Limitations of this research were compounds extracted which are flavonoids and phenolic compounds. Only three types of extraction methods were used which are ultrasonic-assisted extraction, microwave-assisted extraction and maceration.

1.5 Significance of Study

Phenolic compounds had been found to have potential health benefits that were believed to arise mainly from their antioxidants activity. Phenolic compounds act as antioxidants by free radical-scavenging, oxygen radical absorbance, and chelating of metal ions (Halliwell *et al.*, 1995). While flavonoids possessed wide range of biological activities, such as antiallergic, anti-inflammatory, antiviral, antiproliferative and anticarcinogenic activities (Ren *et al*., 2003). The comprehensive study highlighted that flavonoids possess various clinical and pharmalocogical properties which are antidiabetic, antiatherosclerotic, hepato- and gastro-protective, antitumour, antithrombogenic, antiosteoporotic, and antiviral effects. This study made use of *Orthosiphon stamineus* which existed abundantly and could be cultivated easily to obtain phenolic and flavonoids compounds. Once the best extraction technique was figured out, conventional technique to extract phenolic and flavonoids compounds could be replaced.

CHAPTER 2

LITERATURE REVIEW

2.1 *Orthosiphon stamineus*

2.1.1 Introduction

Orthosiphon stamineus or Cat's Whiskers or "misai kucing" is one of the locally well-known herbs and normally distributed at Southeast Asia such as Malaysia, Indonesia, Thailand, Vietnam, and Myanmar. *Orthosiphon stamineus* is perennial herb from Lamiaceae family. Normally, its height is between 0.3 to 1 m and has 4-angled stem. Its flower is white or pale lilac coloured. And its stamens exceed more than 2 cm from the corolla-tube. The leaves have wide 2 to 4 cm, long 4 to 7 cm, egg-shaped spurs, sharp jagged edges rough and irregular, and usually arranged in opposite pairs. It is given common name as "misai kucing" or Cat's Whiskers because of its flowers with long wispy stamens shaped like cat whiskers (Indu and Ng, 2000).

Figure 2.1 White *Orthosiphon stamineus*

Figure 2.2 Purple *Orthosiphon stamineus*

2.1.2 Medicinal Properties of *Orthosiphon stamineus*

The recent surge of interest in *Orthosiphon stamineus* has led to the isolation of more than 50 components with different biological activities (Hossain and Ismail, 2010). Twenty phenolic compounds isolated from *Orthosiphon stamineus* were nine lipophilic flavones, two flavonol glycosides, nine caffeic acid derivatives and the new compound 5, 6, 7, 8-tetrahydroxy-6-methoxy flavones (Amzad Hossain *et al*., 2007). The major part of this plant used for medicinal purposes is its leaves. It was reported that polymethoxylated flavones, sinensetin, tetramethylscutellarein and 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone were present in *Orthosiphon stamineus* leaves.

2.1.3 Phenolic Compounds

Phenolic compounds such as lipohilic flavones, caffeic acid derivatives (rosmarinic acid and 2, 3-dicaffeoyltartaric acid), eupatorine, cichoric acid, sinensetin and methoxy flavones were found in *Orthosiphon stamineus* (Olah *et al*, 2003: Pietta *et al*, 1991). Phenolic substances are the main phytochemicals with antioxidant properties found in plants. These bioactive compounds inhibit lipid autoxidation by acting as radical scavengers and act as essential antioxidants that protect the propagation of the oxidative chain. The intake of phenolic compounds is inversely correlated with the risk of coronary heart disease. These phytochemicals provide health benefits by several mechanisms: free-radical scavenging, protection and regeneration of other dietary antioxidants and chelating of pro-oxidant metal ions. The structure of phenolic compounds is a key determinant of their radical scavenging and metal-chelating activity.

A study by Huang and Zheng (2005) reported that Rosmarinic acid showed several bioactivities including anti-bacterial, anti-inflammatory and anti-carcinogenic activities.

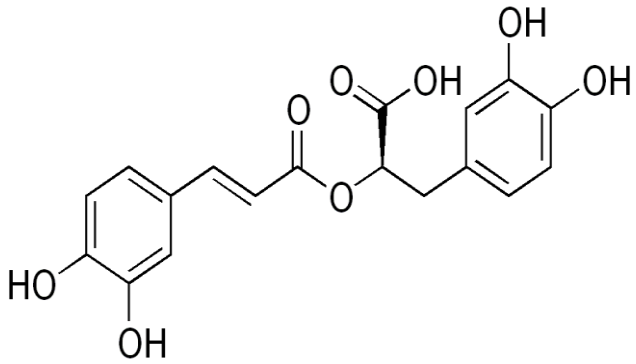

Figure 2.3 Structure of Rosmarinic Acid

2.1.4 Flavonoids Compounds

Flavonoids are large group of secondary metabolites in plants that do not have direct involvement with growth of plants. Flavonoids are biological pigments that provide colours from red to blue in flowers, fruit and leaves. Flavonoids compounds exhibit wide range of biological activities and clinical properties including antimicrobial, antibacterial, anti-inflammatory, anti-allergic and antithrombotic actions (Yao *et al.*, 2004). According to the unsaturation and oxidation degrees of the three-carbon segment, several sub-classes of flavonoids can be distinguished which are flavonols, flavanones, flavones, flavan-3-ols, anthocyanidins and isoflavones (Biesaga, 2011). Flavonoids compounds that can be isolated from *Orthosiphon stamineus* are eupatorin, sinensetin, 5-hydroxy-6,7,3' 4'-tetramethoxyflavone, salvigenin, 6-hydroxy-5, 7, 4'-trimethoxyflavone and 5, 6, 7, 3'-tetramethoxyflavone and 5, 6, 7, 3'-tetramethoxy-4'-hydroxy-8-C-prenylflavones (Hossain and Rahman, 2011). Factors such as place of

plant growth, the genus, extraction conditions and technology contribute to the content of flavonoids compounds (Mao *et al*., 2008). In a study (Arai *et al*., 2000), the total intake of quercetin was inversely related to total cholesterol and low-density lipoprotein (LDL) plasma levels. Sinensetin helps to relax the muscles of the walls of the internal vessels thus facilitating easier flow of urine and even the small particles that become stones. Also, Sinensetin was reported to have high chemo synthesizing effect which was used for the synthesis of the multi- drug resistance cell for anti-cancer drugs (Ahmad *et al*., 2008).

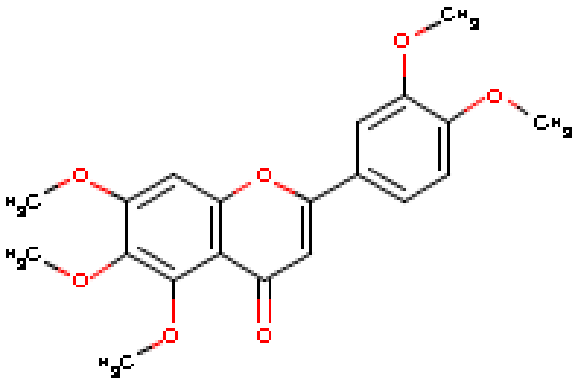

Figure 2.4 Structure of Sinensetin

2.2 Extraction

2.2.1 Introduction

Extraction is a separation process where the distribution of the phenolic compound and flavonoids between two immiscible phases is made in order to achieve the appropriate distribution coefficient. The extraction procedure is sequential and systematically carried out using an aqueous organic solvent to extract phenolic compounds and flavonoids in fruit and vegetables samples. Stability of flavonoids and phenolic compounds will be affected by extraction method.

2.2.2 Ultrasonic-Assisted Extraction (UAE)

2.2.2.1 Introduction

The use of ultrasound to enhance the extraction yield is a technique that started in the 1950s with laboratory scale experiments. The extraction of bioactive compounds under ultrasound irradiation about 20 to 100 KHz offers high reproducibility in shorter times, simplified manipulation, reduced solvent consumption and temperature and lower energy input (Khan *et al.*, 2010). Usually, UAE performed in experimental work is indirect sonication by using a small cleaning bath. Additional agitation or shaking is attached to avoid standing waves or the formation of solid free regions for the preferential passage of the ultrasonic waves. Very few plant materials require more than two hours of sonication. Interestingly, when a process was scaled up in a large ultrasonic cleaning bath, for example 10 litres solvent and over 1000g of plant material, the ultrasonic procedure seems to be a significant improvement when extraction time is taken into account. It is noted that when high frequency ultrasound is employed, the extraction yield did not increase significantly however the degradation of the herb constituents was diminished (Vinatoru *et al*., 1997).

Since vegetal tissue consists of cells surrounded by walls, UAE mechanism involves two types of physical phenomena which are diffusion through the cell walls and rinsing the cell contents once the walls are broken. Both phenomena are significantly affected by ultrasonic irradiation. Some cells exist in the form of internal and external glands that are filled with essential oil. For internal glands, the milling degree of the vegetal material plays important role. Reducing the size of the vegetal

material particles will increase the number of cells directly exposed to ultrasonically induced cavitation. External glands have thin skin and can be easily destroyed by sonication (Vinatoru, 2001). During sonication, cavitation process causes swelling of cells or breakdown of cell walls allows high diffusion rate across cell wall (Vinatoru, 2001).

2.2.2.2 Ultrasonic Cavitation

Ultrasonic cavitation is the momentary creation of “bubbles” in the fluid which immediately and violently implode to produce millions of microscopic jets of liquid which gently scrubs the parts which are submerged in the tank. These cavities are created tens of thousands of times each second to gently remove contaminants without damage, as long as the ultrasonic frequency selected is correct. Ultrasonic cavitation is capable of selectively disrupting the subcutaneous cells through thousands of microscopic implosions impacting the cell membranes. When high power ultrasonic is doing in liquid material media, tens of thousands times ultrasonic vibration will be produced per second continuously, to prompt liquid molecular fast movement.